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Biological activities of three *Phlomis* species

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ABSTRACT: The genus *Phlomis*, which belongs to the Lamiaceae family, comprises 100 species native to Turkey, Asia, Europe, and North Africa. As reported in many studies, *Phlomis* genus has unique therapeutic and aromatic properties. According to the data obtained from traditional uses, *Phlomis* species have a characteristic taste and so are traditionally consumed as herbal tea to treat diabetes, gastrointestinal disease as well as maintain overall health by protecting the liver, kidney, bone, and cardiovascular systems. In this present work, it was focused on the evaluation of the *in-vitro* enzyme inhibition (*a*-glucosidase, *a*-amylase, acetylcholinesterase (AChE)/butyrylcholinesterase (BuChE), and tyrosinase (TYR) and non-enzyme antioxidant activities (TPC, TFC, DPPH, and ABTS radical scavenging assays, and Iron-chelating activity assay) of the dichloromethane (DCM) and methanol extracts prepared from aerial parts of *Phlomis nissolii, P. samia* and *P. sieheana* with ultrasonic assisted method. All methanol extracts were rich in phenolic compounds and have high antioxidant activities. In the literature, there is no activity study related to the extracts of *P. sieheana.* The methanol extract of *P. samia* was the most active among other observed species. Especially the high tyrosinase enzyme inhibition activity of *P. sieheana* DCM extract may be promising for cosmetic product development.

KEYWORDS: *Phlomis nissolii; P. samia; P. sieheana;* antioxidant activity; enzyme inhibitory

1. INTRODUCTION

The Lamiaceae family includes the genus *Phlomis* L., which has 100 species widespread to Europe, Turkey, Asia and North Africa (1). In Turkish flora, 52 species are spread, including 6 variations, 12 natural hybrids, and 34 endemic taxa (2). *P. nissolii* is endemic species, while *P. samia* and *P. sieheana* are a plant commonly found in the Mediterranean region. The Phlomis genus includes perennial herbs and small shrubs that are pilose or tomentose, have glands or not, and have stellate to dendroid hairs (3).

There are a wide variety of ethnobotanical uses that differed by country. Their flowering parts are commonly used as herbal tea to treat gastrointestinal disease as well as to maintain overall health by protecting the liver, kidney, bone, and cardiovascular systems. They're also traditionally used as stimulants, tonics, and diuretics, and they've shown to have biological activities that can treat ulcers, hemorrhoids, and wounds (4). According to the data obtained from traditional uses, *Phlomis* species have a characteristic taste and so can be consumed as herbal tea in folk medicine. Moreover, *Phlomis tuberosa* and *P. fruticosa* have edible uses (1).

Also, many studies have been reported that *Phlomis* genus have distinct fragrance and therapeutic properties (4). The essential oils of the *Phlomis* genus are utilized in the culinary and medicinal applications. Essential oils extracted from *Phlomis* species are utilized in the perfume and cosmetic industries as flavoring and aroma (5). The chemical compositions of essential oils of *Phlomis* are found as α-pinene, linalool and limonene (monoterpenes), *β*-caryophyllene and germacrene D (sesquiterpenes), hexadecanoic acid (fatty acid) aliphatic components, and other compounds. Phenylethyl alcohol, polyphenolics and iridoids are major compounds isolated from *Phlomis* extracts prepared with different solvents. Luteolin, apigenin, chrysoeriol, kaempferol, eriodictyol, naringenin, and their glycosides are the main flavonoids identified in the *Phlomis* species (1).

Previously, literature has been investigated on the iridoid and phenylpropanoid components of *Phlomis samia* and iridoid glucosides such as lamiide, shanzhiside methylester, and ipolamiide was identified (6).

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Verbascoside is the main compound in the extract obtained from the aerial parts of the *P. nissolii*, and also found isoverbascoside, forsythoside B, samioside, and alyssonoside belonging to the phenylethanoid glycoside group (7-8).

The biological properties of some *Phlomis* species have been investigated and they are described for antinociceptive, antidiabetic, anti-inflammatory, anticancer, antioxidant, and antimicrobial activities (1).

Members of the genus *Phlomis* are worth investigating owing to their unique traditional usage or scientifically proven biological properties. In this present work, it was focused on the evaluation of the *in-vitro* enzyme inhibition and non-enzyme antioxidant activities of the dichloromethane and methanol extracts prepared from herbs of three species, which are *P. nissolii* L., *P. samia* L., and *P. sieheana* Rech. fil. with ultrasonic assisted method (1, 6).

The objectives of this research were to: [1] evaluate and compare the *in vitro* antioxidant activities of various extracts from *P. nissolii, P. samia* and *P. sieheana* by three bioassays (DPPH and ABTS radical scavenging assay, Iron chelating effect); [2] quantify total phenolic compounds and total flavonoids; [3] antidiabetic activity using *a*-amylase and *a*-glucosidase enzyme inhibition assays; [4] acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities; [5] inhibition activity of the tyrosinase enzyme.

2. RESULTS AND DISCUSSION

2.1. Yields of plant extracts

In the present work, the extracts were prepared from aerial parts of *Phlomis nissolii*, *P. samia* and *P. sieheana* with ultrasonic assisted method. Dichloromethane and 80% methanol were used for the extraction. The yields of the extracts are given in Table 1. The yields of DCM extracts are lower than that of the methanol extracts.

Table 1. Yields of DCM and methanol extracts of *Phlomis* species

Plant	Dichloromethane extract yield %	80% Methanol extract yield %
Phlomis nissolii	1.36	18.22
Phlomis samia	4.33	23.69
Phlomis sieheana	3.97	24.48

2.2. Determination of antioxidant properties of *Phlomis* species

Antioxidant activity of DCM and 80% methanol extracts of aerial parts of *Phlomis* species was assessed using ABTS and DPPH radical scavenging, and iron chelating methods, as well as Total Phenol/Flavonoid Content (TPC/TFC) measurements. The TPC and TFC of *P. nissolii, P. samia* and *P. sieheana* were given in Figure 1. According to the analysis results, TPC results were found higher than TFC in methanol extracts of all species. Among these extracts, the highest phenolic compound (175.72 mg GAE/g extract) and has the total flavonoid content (89.25 mg QE/g extract) were found in the methanol extract of *P. sieheana*. The DCM of *P. samia* extract, which is among all the extracts, has the highest TFC.

In a previous study, *P. samia* was collected from Tunisia. The methanol extract of aerial parts of *P. samia* was investigated and TPC value was found 73.14 mg GAE/g extract and TFC value is 21.61 mg QE/g extract. In addition, its antioxidant activity was examined by the DPPH method (9). Their results were higher than the values obtained in our study. The reason for this may be that the prepared extract is 80% methanol, and the plant is harvested at different locations and times. In a separate investigation, samioside, isolated from the methanol extract of *P. samia,* was found as effective DPPH scavengers. The other phenolic compounds such as phenylethanoid glycosides and flavonoids could be responsible for the activity in our investigation (10).

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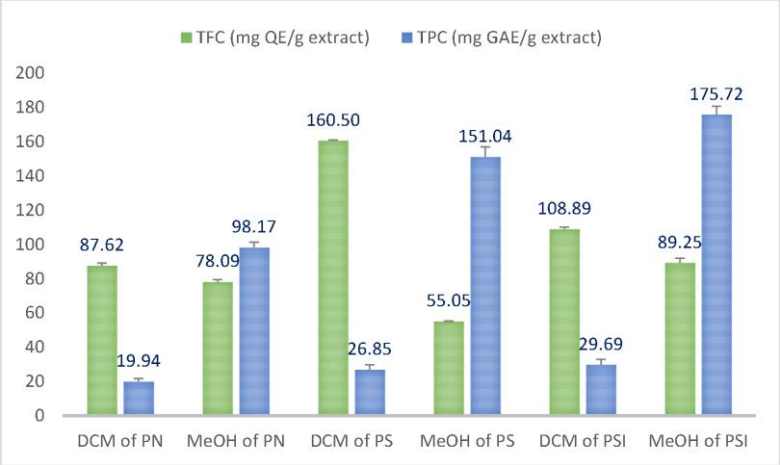

Figure 1. TPC and TFC of *Phlomis* species

There are various antioxidant mechanisms such as radical scavenging, iron chelation, and peroxidation delay. In this study, antioxidant mechanisms of DCM and methanol extracts of *Phlomis* species were elucidated by DPPH, ABTS and iron chelating methods. *In vitro* antioxidant activities (%) of DCM and methanol extracts of *Phlomis* species are summarized in Table 2. The samples with the highest antioxidant activity in all three different methods used were methanol extracts. The effect of DCM extracts was very weak or not observed. The methanol extract of *P. sieheana* had more antioxidant potential than other species. The antioxidant activity findings were demonstrated similar profile with the total phenolic compound results. It was indicated that phenolic compounds may be responsible for antioxidant activity. As a result, it is possible that the polar components of methanol extracts are mainly responsible for their free radical scavenging capability. Previously DPPH antioxidant activity (EC_{50}=32µg/mL) study was conducted with the leaves of *P. samia* collected from Algeria (11). There is no study on *P. sieheana* for its antioxidant activity in literature. On the other hand, the antioxidant activity of *P. nissolii* was shown by Sarıkürkçü et al. The radical scavenging capability of this plant by DPPH, ABTS, and superoxide radical methods was assessed in this study. The biological capabilities of aromatic and/or medicinal plants appear to be linked to phenolic components, specifically phenolic acids and flavonoids. So, TPC and TFC have also shown to be effective within the study (3).

Table 2. *In vitro* Antioxidant Activities (%) of DCM and methanol extracts of *Phlomis* species

SAMPLE	ABTS (TEAC±S.D.[a])	DPPH (percentage±S.D.[a]) 100 µg/mL	Iron chelating effect (percentage±S.D.[a]) 250 µg/mL
DCM of PN	188.73±3.69 [b]	n.e.	49.10±0.09
MeOH of PN	536.19±0.81 [b]	49.69±0.64	55.80±0.78 (IC_{50}: 93.59±0.12)
DCM of PS	84.40±1.01 [b]	n.e.	63.24±0.10 (IC_{50}: 133.45±3.89)
MeOH of PS	533.59±0.55 [b]	78.43±0.20 (IC_{50}: 50.17±0.08)	65.28±0.42 (IC_{50}: 165.40±3.96)
DCM of PSİ	54.16±4.00 [b]	n.e.	59.48±0.35 (IC_{50}: 84.30±0.79)
MeOH of PSİ	517.13±0.46 [b]	75.08±0.06 (IC_{50}: 82.77±0.04)	63.28±0.85 (IC_{50}: 105.50±0.57)
Reference[b.c.d]	86.95 ± 1.26[c]	91.81 ±0.04[d]	87.06±0.34 [e]

a: standard deviation,
b: Trolox equivalents at 1 mg/ml concentration,
c: Trolox,
d: Gallic acid,
e: EDTA, **n.e.:** not effected,
PN: *P. nissolii*, **PS:** *P. samia*, **PSI:** *P. sieheana*

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2.3. Enzyme inhibition activities

As for the enzyme inhibitory activity on this work, the inhibitory effects of DCM and MeOH extracts of *Phlomis* species aerial parts on α-glucosidase, α-amylase, acetylcholinesterase (AChE)/butyrylcholinesterase (BuChE), and tyrosinase (TYR) of *P. nissolii, P. samia* and *P. sieheana* were given in Table 3.

In the tyrosinase enzyme inhibition study, the most active results were observed in DCM extracts of *Phlomis* species. In this study, the activity of DCM extract of *P. nissolii* was lower than the other species and kojic acid. Previously, different solvent extracts of *P. nissolii* were investigated for antioxidant activity. The aqueous and ethyl acetate extracts of *P. nissolli* outperformed in antioxidant activity than the methanol extract (3). The results obtained in this study were supported by our study with the methanol extract of *P. nissolii.* The enzyme inhibition activities of *P. nissolli* extracts were also tested against AChE, BuChE, α-glucosidase, and α-amylase. *P. nissolli* demonstrated exceptional inhibitory capacity in enzyme inhibition experiments. Ethyl acetate extract, in particular, had a higher inhibitory activity (4). The *P. samia* and *P. sieheana* have not researched previously.

In our study, while the DCM and methanol extract of *P. samia* and *P. sieheana* had strong inhibitory activity against AChE and BuChE, the DCM extract of *P. nissolii* had no activity. The cholinesterase inhibitory effect of DCM and methanol extracts might be observed due to the phytochemical components. Extracts had showed the greatest cholinesterase inhibition effect among the enzymes investigated in our study. As a result, the extracts of *P. nissolii, P. samia* and *P. sieheana* tested in our study might be useful for the discovering natural agents for the treatment of neurological disorders.

A few *Phlomis* species have been identified as having antidiabetic activity. In this context, antidiabetic activities of *Phlomis aurea, P. ocymifolia,* and *P. anisodonta* have been investigated until now (1, 12). Their potential effects to protect liver and pancreas integrity by lowering oxidative stress in diabetes or promoting the synthesis of enzymes involved in glucose metabolism may be the main reason for their activity. The enzymes α-glucosidase and α-amylase are associated with antidiabetic effects. The results are given in **Table 3.** Consequently, *P. nissolii* extracts showed no effect against *a*-glucosidase and *a*-amylase enzymes. The enzyme inhibitory activities of DCM and methanol extracts of *P. samia* were determined as the highest species among the tested *Phlomis* species.

Table 3. Enzyme inhibition activities of *P. nissolii, P. samia* and *P. sieheana*

SAMPLE	Inhibitory activity against TYR (percentage±S.D.[a])	Inhibitory activity against AChE (percentage ± S.D.[a])	Inhibitory activity against BuChE (percentage±S.D.[a])	Inhibitory activity against alpha glucosidase (percentage ±S.D.[a])	Inhibitory activity against alpha amylase (percentage ±S.D.[a])
DCM of PN	39.51±0.04	49.43±0.9[b] (IC_{50}: 74.52±3.32)	n.e.	n.e.	n.e.
MeOH of PN	11.96±1.82	68.64±0.53 (IC_{50}: 114.45±1.33)	47.84±0.5[b] (IC_{50}: 93.33±0.01)	n.e.	n.e.
DCM of PS	46.07±1.90	90.02±2.57 (IC_{50}: 54.37±2.35)	67.67±6.5[b] (IC_{50}: 28.15±2.06)	21.43±2.13[c]	16.38±3.5[d]
MeOH of PS	19.51±1.96	71.38±0.23 (IC_{50}: 75.81±0.60)	82.76±1.9 (IC_{50}: 53,005±1.58)	48.01±1.8[c]	13.32±2.6[d]
DCM of PSİ	47.71±1.20	68.64±0.53 (IC_{50}: 94.13±0.96)	86.21±0.9 (IC_{50}: 67,6125±3.24)	17.37±0.82[c]	3.74±0.71[d]
MeOH of PSİ	18.73±0.81	68.64±0.53 (IC_{50}: 72.93±0.26)	53.88 0.5 [b] (IC_{50}: 74,295±3.39)	29.89±4.86[c]	4.59±4.6[d]
Reference[f,g,h]	80.96 ±0.51[e]	99.10±1.18[f]	84.34±4.85[f]	53.89±3.24[g]	50.65±0.86[g]

a: standard deviation,
b: 100µg/ml, **c:** 80µg/ml, **d:** 20µg/ml **e:** Kojic acid (250µg/ml), **f:** Galantamin (200 µg/ml), **g:** Acarbose (160 µg/ml for *a*-glucosidase and 250µg/ml for *a*-amylase),
n.e.: not effected,
PN: *P. nissolii,* **PS:** *P. samia,* **PSI:** *P. sieheana,*
TYR: Tyrosinase, **AChE:** Acetylcholinesterase, **BuChE:** Butyrylcholinesterase.

Although there are previous studies on the essential oil of *P. sieheana,* there is no biological activity assessment on the extracts of *P. sieheana.* The antioxidant activity of the *P. samia* plant was investigated before. However, antidiabetic activity of *P. samia* was examined for the first time in our study. It was reported that the most active findings among the species was determined in the methanol extract of *P. samia*. In addition, the extracts of *P. nissolli* exhibited consistent findings with previous researches (4).

3. CONCLUSION

According to our findings, methanol extracts of the *Phlomis nissolii, P. samia* and *P. sieheana* showed considerable against oxidative agents. In addition, our research suggests that the extracts of these *Phlomis* species can be used as natural antioxidants in the food and pharmaceutical industries, filling the gap in the literature. It is also worth noting that the efficacy in the treatment of neurological disorders is particularly high. Therefore, its phytochemical structure should be clarified, and *in vivo* and clinical studies should be proceeded in the future.

4. MATERIALS AND METHODS

4.1. Plant materials

The aerial parts of three *Phlomis* species were collected in Turkey. Dr. Yavuz Bağcı and Dr. Süleyman Doğu identified the plant materials. The materials were kept at the Herbarium of Selçuk University in Konya (KNYA), Turkey. The herbarium codes, collecting times and locations of the plants are given in Table 4.

Table 4. The herbarium codes, collected date and locations of the *Phlomis* species

Plant	Location	Attitude	Date	Herbarium code
Phlomis nissolii (PN)	Karaman	1000m	July, 2018	S. Doğu 3088
Phlomis samia (PS)	Antalya	670m	Jun, 2018	S. Doğu 3065
Phlomis sieheana (PSİ)	Aksaray	1050m	July, 2018	S. Doğu 3117

4.2. Ultrasonic assisted method

The aerial parts of three *Phlomis* species were dried in the shade and the dry stems and leaves were crushed in a blender. The powdered plant material was then extracted in the ultrasonic bath with Dichloromethane (Sigma Aldrich) and Methanol (80%, v/v) (Sigma Aldrich) at room temperature for 24 hours. Extracts were eventually filtered using filter paper after the maceration step was repeated three times. The combined filtrates were concentrated with a rotary evaporator (Buchi R300) to dryness under reduced pressure at 40°C. The dried extracts were then kept at -20°C until they were tested for antioxidant and enzyme inhibition properties. The yields of obtained extracts are given in Table 2.

4.3. Determination of antioxidant properties of *Phlomis* species

The antioxidant properties of DCM and methanol extracts of aerial parts of three *Phlomis* species were investigated using several assays, including total flavonoid and phenol content, DPPH, $ABTS^{\bullet+}$ radical scavenging activities, and Fe^{+2} chelating.

4.3.1. Total phenolic content determination (TPC)

The spectrophotometric Folin-Ciocalteu (F-C) technique was used to measure the TPC in the DCM and methanol extracts, with minor modifications to Clarke et al. method (13). In summary, 10 µl of extract was mixed with 100 µl of newly diluted F-C reagent in distilled water. After 5 minutes, the solution was combined with 100 µl of 7.5 percent Na_2CO_3 solution and incubated at room temperature for 60 minutes. In a microplate reader, the absorbance was measured at 650 nm. All analyses were done in triplicate, and the findings were presented as mean ± standard deviation (SD). The TPC was determined as mg GAE (gallic acid equivalent) g extract (Sigma-Aldrich) using the calibration curve of concentration vs. absorbance.

4.3.2. Total flavonoid content determination (TFC)

The aluminum chloride colorimetric technique was used to estimate TFC in the DCM and methanol extracts of three *Phlomis* species (14). In a 96-well plate, 150 µl of extract sample (0.3 mg/ml) dissolved with ethanol were combined with 150 µl of 2% $AlCl_3$ (Sigma-Aldrich). In a microplate reader, the absorbance was measured at 435 nm after 15 minutes of room temperature incubation. Triplicates were used for all calculations. On a dry weight basis, TFC was determined as mg QE (quercetin equivalent)/g extract (Sigma-Aldrich).

4.3.3. Radical scavenging activity of DPPH•

The effects of the DCM and methanol extracts on free radical scavenging were investigated using the Clarke et al. technique. In 96-well plates, 20 µl of extract were combined with 180 µl of newly prepared 0.04 mg/ml DPPH• (Sigma-Aldrich) methanol solution. The negative control was methanol solvent, while the positive control was gallic acid. The plate was measured at 540 nm with an Elisa reader (Multiscan Sky, USA) after 15 minutes of incubation at 25°C in the dark (13).

4.3.4. Radical scavenging activity of ABTS•+

According to Re et al. the procedure was followed with minor modifications to determine the ABTS•+ radical scavenging properties of the DCM and methanol extracts (15). 7 mM ABTS•+ (Sigma-Aldrich) solution was mixed with 2.4 mM potassium persulfate solution in equal volume for 16 hours in the dark to make the ABTS•+ stock solution. The studying solution was then made by diluting the stock ABTS•+ until it has an absorbance of 0.70±0.02, then mixing 50 µl of extract with 100 µl of ABTS•+ test solution and leaving it for 10 minutes. At 734 nm, the plate was measured. All the activities were repeated three times, with the results reported as the mean ± standard deviation. Appropriate negative standard (ethanol) and positive control (trolox) (Sigma-Aldrich) were analyzed simultaneously. The percent inhibition of test samples was computed using the following equation 1:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test sample})}{\text{Absorbance of control}} \times 100 \quad \text{(Eq. 1)}$$

4.3.5. Iron chelating activity

The interaction of the DCM and methanol extracts with the Fe^{2+}-ferrozine complex was used to determine their iron chelating activity. 50 µl of extract were combined with 50 µl of $FeSO_4$ (0.1 mM) (Sigma-Aldrich) and 100 µl of sodium salt of 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid (Sigma-Aldrich) (16). The absorbance was recorded at 562 nm after 10 minutes incubation period. As a positive control, EDTA was applied.

4.4. Enzyme inhibition activities

4.4.1. Inhibition activity of the tyrosinase enzyme

The tyrosinase enzyme inhibition test was carried out according to Yang et al. process (17). The extracts combined with 100 µl of phosphate buffer (0.1 M, pH = 6.8) and 20 µl of phosphate buffer (0.1 M, pH = 6.8). The plate was incubated for about 10 minutes at 25°C after adding 20 µl of tyrosinase (250 U/ml) (Sigma-Aldrich). Then, as a substrate, 20 µl of 3 mM L-tyrosine was added and incubated for another 30 minutes at 25°C. At 492 nm, the absorbance was measured. As a positive control, kojic acid (Acros) was applied. Each sample was run in three parallels with five different concentrations. The inhibitory rate of extracts on tyrosinase was determined using the equation 1.

4.4.2. Acetylcholinesterase (AChE)/Butyrylcholinesterase (BuChE) inhibition assay

This test was carried out in accordance with the procedure by Paşayeva et al (18). For 14 minutes, a mixture of 20 µl of extract sample/positive control at various concentrations, 20 µl of enzyme (0.22 U/ml for AChE, and0.1 U/ml for BuChE) (Sigma-Aldrich), and 140 µl of 0.1 mM phosphate buffer (pH: 6.8) were incubated with 140 µl of 0.1 mM phosphate buffer (pH: 6.8). Following the addition of 10 µl of 0.5 mM DTNB (Sigma-Aldrich), 10 µl of substrate (0.2 mM butyrylthiocholine iodide/ 0.71 mM acetylthiocholine iodide) (Sigma-Aldrich) was mixed and incubated for another 5 minutes. When the substrate was applied, the

mixture's absorbance was measured at 0 and 5 minutes, resulting in a yellow color measurement of 412 nm. As a positive control, galantamine (Sigma-Aldrich) was employed (Eq.2).

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control}_{0-5\text{min}} - \text{Absorbance of test sample}_{0-5\text{min}}}{\text{Absorbance of control}_{0-5\text{min}}} \times 100 \text{ (Eq.2)}$$

4.4.3. α-glucosidase inhibition assay

The DCM and methanol extracts were tested for α-glucosidase inhibitory activity using the chromogenic technique described by Salehi et al. (19). 120 µl of 0.1 M phosphate buffer (pH 6.9), 10 µl of test material (concentration: 0-2 mg/ml), and 20 µl of *α*-glucosidase (0.5 U/ml) (Sigma-Aldrich) at various concentrations were combined in 96-well plates., the mixture was incubated for 15 minutes at 37°C. The enzymatic reaction was started by adding 20 µl of 5 mM *p*-nitrophenyl-D-glucopyranoside (Sigma-Aldrich) solution to the reaction mixture after preincubation, and incubated for additional 15 minutes at 37°C. After adding 80 µl of 0.2 M sodium carbonate solution to stop the reaction, the absorbance was measured at 405 nm using a microplate reader. The inhibitory rate of extracts on *α*-glucosidase was determined using the equation 1.

4.4.4. α-amylase inhibition assay

For screening the DCM and methanol extracts of *Phlomis* species for antidiabetic potential, the method of Lalitha et al. for *α*-amylase (Sigma-Aldrich) inhibition test was modified (20). The starch solution (0.1% w/v) was prepared freshly (100 mg of potato starch was heated for 15 minutes in 100 mL of 20 mM sodium phosphate buffer (pH 6.9) with 6.7 mM sodium chloride). The coloring reagent was produced with sodium potassium tartrate (12 g) mixed in sodium hydroxide (8 ml, 2 M) and 3,5-dinitrosalicylic acid (96 mM) while stirring. The plant extracts (20 µl) and starch solution (1 ml) were incubated at 20°C for 20 minutes at first, and then for 3 minutes following the addition of **α**-amylase (1 ml). After adding 1ml of coloring reagent, the solution was heated in a water bath at 75-80°C for 15 minutes. The produced color was measured at 540 nm. The % inhibition of *α*-amylase inhibitory activity was determined using the equation 3.

$$\% \text{ Inhibition} = \frac{[Absorbance\ of\ control - (Absorbance\ of\ test\ sample - Absorbance\ of\ control)]}{Absorbance\ of\ control} \times 100 \quad \text{(Eq.3)}$$

5. STATISTICAL ANALYSIS

The mean and standard deviation of data from enzyme inhibition and *in vitro* antioxidant tests were calculated (SD). The experiments were done in triplicates and all data were shown as mean ± standard deviation (SD). GraphPad Software Prism 8 (San Diego, CA, USA) was used to determine the % inhibitory impact of the extracts and standard compounds based on the extracts dose-response curve.

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